

Ag N — cut one at Nde I to see if all of loc is present even though Aat II is missing

Aat II Nde I — R21 Aat III
 Line 267 expect 677 bp Nde I / Aat III band

Nde II is OK in buffer 4 (NEB) (P109)

I cut with Sst I, Kpn I or Bam H I to see if missing R1 site is only a point mutation (and OK in NEB buffer 4)

Aat II R21 Sst I Kpn I Bam H I (for colonies #20 (tubes 2, 4, 6 below) see if Sst I, Kpn I or Bam H I can cut in MCS)

TA #1 5 5 5 5 ✓
 (blue colony)
 (missing R1)
 missing Aat II 5 5 5 ✓
 ✓

Sst I Kpn I Bam H I

R1 0.1 → - ✓
 P1 III 0.5 → ✓
 I 1 1 ✓
 n 1 1 1 ✓
 Bam H I 1 1 ✓
 R1 1 1 ✓
 NEB 11.6 → ✓
 B buffer 4 2 → ✓
 20 μl



37°C 2 hr

Result

G A A T T C G A G C T C

Sst I cuts but R1 did not (P106)

R1 — Sst I

so mutation is no more than 1 bp downstream from the R1 site and may even be a point mutation

677 bp

Aat II Nde I Kpn I Bam H I
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Nde may give the 677 bp fragment expected but multiple bands are seen

ssed & Understood by me,

Date

Constance Bolao

2/10/95

Invented by

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1/13/95